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Review article

The preterm parturition syndrome

R Romero,[a] J Espinoza,[a] JP Kusanovic,[a] F Gotsch,[a] S Hassan,[a] O Erez,[a] T Chaiworapongsa,[b] M Mazor[c]

[a] Perinatology Research Branch, NICHD, NIH, DHHS, Bethesda, Maryland and Detroit, MI, USA [b] Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA [c] Department of Obstetrics and Gynecology, Soroka Medical Center, Ben Gurion University, Beer Sheva, Israel

Correspondence: Dr Roberto Romero, Perinatology Research Branch, NICHD/NIH/DHHS, Hutzel Women's Hospital—Box #4, 3990 John R, Detroit, MI 48201, USA. Email awarfiel@med.wayne.edu

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The implicit paradigm that has governed the study and clinical management of preterm labour is that term and preterm parturition are the same processes, except for the gestational age at which they occur. Indeed, both share a common pathway composed of uterine contractility, cervical dilatation and activation of the membranes/decidua. This review explores the concept that while term labour results from physiological activation of the components of the common pathway, preterm labour arises from pathological signalling and activation of one or more components of the common pathway of parturition. The term 'great obstetrical syndromes' has been coined to reframe the concept of obstetrical disease. Such syndromes are characterised by: (1) multiple aetiology; (2) long preclinical stage; (3) frequent fetal involvement; (4) clinical manifestations that are often adaptive in nature; and (5) gene–environment interactions that may predispose to the syndromes. This article reviews the evidence indicating that the pathological processes implicated in the preterm parturition syndrome include: (1) intrauterine infection/inflammation; (2) uterine ischaemia; (3) uterine overdistension; (4) abnormal allograft reaction; (5) allergy; (6) cervical insufficiency; and (7) hormonal disorders (progesterone related and corticotrophin-releasing factor related). The implications of this conceptual framework for the prevention, diagnosis, and treatment of preterm labour are discussed.

Keywords Allergy, cervical insufficiency, inflammation, intrauterine infection, multiple aetiology, prematurity, preterm birth, preterm labour, uterine ischaemia, uterine overdistension.

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Introduction

The implicit paradigm that has governed much of the study of preterm parturition is that term and preterm labour are fundamentally the same process except for the gestational age at which they occur[1,2] and share a 'common pathway'. The uterine components of this pathway include increased uterine contractility, cervical ripening (dilatation and effacement), and decidua/membrane activation.[2,3]

Nearly two decades ago, our group proposed that the fundamental difference between term and preterm parturition is that the former results from physiological activation of the common pathway, while preterm labour arises from pathological processes that extemporaneously activate one or more of the components of the common pathway of parturition. This article will review the evidence that preterm labour is a pathological condition with multiple aetiologies. This has implications for the fundamental understanding of the biology of preterm parturition and the clinical strategies to diagnose, prevent, and treat spontaneous preterm labour.[1,4] Some of these concepts were presented at the Premature Labour Study Group convened by the Royal College of Obstetricians and Gynaecologists and published in a contribution to the proceedings, as well as in a previously published book chapter[5,6]

The common pathway of parturition: definition and components

We propose that the common pathway of human parturition be defined as the anatomical, physiological, biochemical, endocrinological, immunological, and clinical events that occur in the mother and/or fetus in both term and preterm labour. The common pathway of parturition is particularly evident when examining the uterine components. Parturition is accompanied by profound changes in other organ systems, and these are the extrauterine components to the common pathway. Similarly, fetal physiopathologic adaptations associated with impending spontaneous birth are likely to occur, such as modifications in lung water distribution.[7] These changes

are difficult to study in humans, and most of the literature is confined to animal studies.

The uterine components include: (1) increased myometrial contractility; (2) cervical ripening (dilation and effacement); and (3) decidual/membrane activation. Examples of non-uterine features of the common pathway include changes in the concentrations of hormones such as corticotrophin-releasing factor (CRF) and cortisol, and in the caloric metabolic expenditures.[8–17]

The common pathway can be defined at different levels of complexity. The definition used above is based on a clinical perspective. A molecular and physiological approach to this definition could use high-dimensional biological techniques to describe the changes in messenger RNA (mRNA), proteins, metabolites, physiological parameters, etc., which occur during labour. We anticipate that transcriptomics (functional genomics), proteomics, metabolomics, physiomics, etc., will be used for a comprehensive description of the common pathway in the future.[18,19] This approach has merit since the dissimilarities between term and preterm birth will provide insights into the mechanisms of disease responsible for preterm parturition. We have begun this process by examining the transcriptome of the chorioamniotic membranes in spontaneous labour at term[20] and in preterm labour with and without inflammation (R Romero, unpubl. obs.).

For a comprehensive description of the common pathway of parturition, the reader is referred to other reviews in this area, in particular, to the proceedings of the Preterm Birth Study Group of the Royal College of Obstetricians and Gynaecologists.[5] The proceedings contain learned discussions of each of the components of the pathway by experts in each particular field (Professors Bell, Norman, Calder, Bennett and Thornton).

Premature parturition: a syndrome

The current taxonomy of disease in obstetrics is based on the clinical presentation of the mother and not on the mechanism of disease responsible for the clinical manifestations. The term 'preterm labour' does not indicate whether the condition is caused by infection, a vascular insult, uterine overdistension, an abnormal allogenic recognition, stress, or some other pathological process. The same applies to pre-eclampsia, small for gestational age, fetal death, nausea and vomiting during pregnancy, and failure to progress in labour, in which the diagnoses simply describe the clinical manifestations without consideration of the specific aetiology.

The lack of recognition that these conditions simply represent a collection of signs and symptoms with little reference to the underlying mechanisms of disease may be responsible for the expectation that one diagnostic test and treatment will detect and cure each of these conditions.

We have proposed that the term 'syndrome' is more apt to refer to the previously mentioned obstetrical disorders. The Oxford Medical Dictionary defines a syndrome as 'a combination of symptoms and/or signs that form a distinct clinical picture indicative of a particular disorder'. Implicit in this definition is that a syndrome can be caused by more than one mechanism of disease or aetiology.

We have argued that obstetric disorders responsible for maternal death and perinatal morbidity and mortality are syndromes, hence, the designation of 'the great obstetrical syndromes'. Key features of these syndromes[21] are: (1) multiple aetiology; (2) long preclinical stage; (3) frequent fetal involvement; (4) clinical manifestations which are often adaptive in nature; and (5) predisposition to a particular syndrome is influenced by gene–environment interaction and/or complex gene-gene interactions involving maternal and/or fetal genotypes.

This article will review the available evidence to support the concept that premature parturition has 'multiple aetiologies'. However, preterm parturition meets all the criteria for a great obstetrical syndrome. For example, a sonographically short cervical length in the mid-trimester of pregnancy or high concentrations of fetal fibronectin in vaginal/cervical fluid are risk factors for subsequent spontaneous preterm labour and preterm birth.[22–27] Since a short cervix or a positive fetal fibronectin generally occur weeks before the clinical recognition of spontaneous preterm labour and/or preterm prelabour rupture of membranes (PPROM), this can be taken as evidence that there is a subclinical stage in which pregnant women have abnormalities that may not be detected by standard clinical examination: a long preclinical stage. This also applies to intrauterine infection which can be clinically silent weeks or months before the onset of preterm parturition. Such infections have been detected at the time of routine mid-trimester amniocentesis for genetic indications in 0.4% of women and become clinically evident weeks later as either PPROM or preterm labour.[28–30] 'Fetal involvement' in the context of infection has been shown in women with microbial invasion of the amniotic cavity (MIAC). Fetal bacteraemia has been detected in 30% of women with PPROM and a positive amniotic fluid (AF) culture for microorganisms.[31] Similarly, neonates born after spontaneous preterm labour or PPROM are more likely to be small for gestational age, indicating a pre-existing problem with the supply line, which results in fetal involvement.[32–37] The 'adaptive nature' of the clinical manifestation has been proposed in the context of preterm labour with intrauterine infection. The onset of preterm labour can be considered a mechanism of host defence against intrauterine infection whereby the mother eliminates infected tissues (membranes, decidua, and/or fetus) to maintain reproductive fitness. When the fetus is mature, the onset of premature labour may also have survival value for it allows the fetus to escape a hostile intrauterine environment. The complexity of nature's

calculation to balance maternal and fetal interests in this context cannot be overemphasized.[38,39] It is possible that other mechanisms of disease in preterm labour may also threaten the maternal and fetal pair (i.e. ischaemia/haemostatic disorders) and a key question would be why some hosts resort to fetal growth restriction, others to pre-eclampsia, and yet others to the onset of preterm labour to deal with the underlying insult. If the clinical manifestations are adaptive, then treatment of the components of the terminal pathway (tocolysis, cerclage, etc.) could be considered as symptomatic and not aimed at the specific pathological process that causes preterm labour. Finally, the predisposition to use a specific mechanism of host defence (e.g. PPROM or preterm labour with intact membranes) may be determined by a 'gene-environment interaction' or 'gene-gene interactions' as in other complex disorders. Complexity is added during pregnancy by the presence, and even perhaps the conflicting interest, of two genomes (maternal and fetal).

The pathological processes implicated in the preterm birth syndrome include intrauterine infection, uterine ischaemia, uterine overdistension, abnormal allogenic recognition, allergic-like reaction, cervical disease, and endocrine disorders (Figure 1). The possibility that mechanisms of disease not yet described may be operative must be considered. Most of the understanding of the mechanisms of disease in obstetrics has been derived from observations in adults and children. The biology of pregnancy is unique since it requires the pacific co-existence of two hosts. The challenges presented by this intimate relationship could create conditions in which novel mechanisms of disease may emerge. Normal pregnancy is characterised by bi-directional traffic of cells (maternal and fetal). Increased fetal DNA has been reported in the maternal blood of women in preterm labour, leading to preterm birth.[40,41] It is possible that abnormal fetal–maternal cell traffic poses challenges that can only be resolved, in some women, with preterm labour. Why excess fetal traffic is associated with pre-eclampsia[42–45] in some women, and to preterm labour[40,41] in others, is unclear. The following sections will review the evidence supporting different mechanisms of disease in preterm labour.

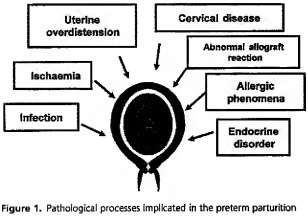

Figure 1. Pathological processes implicated in the preterm parturition syndrome. (Reproduced with permission from reference 5.)

Infection as a cause of preterm labour

Intrauterine infection has emerged as a frequent and important mechanism of disease in preterm birth.[46–49] It is the only pathological process for which a firm causal link with preterm birth has been established and for which a defined molecular pathophysiology is known.[3]

Evidence of causality

The evidence in support of a causal relationship between infection/inflammation and spontaneous preterm labour includes: (1) intrauterine infection or systemic administration of microbial products to pregnant animals can result in spontaneous preterm labour and preterm birth;[47,50–62] (2) extrauterine maternal infections, such as malaria,[63–66] pyelonephritis,[67–71] pneumonia,[72–74] and periodontal disease,[75–80] have been associated with preterm birth; (3) subclinical intrauterine infections are associated with preterm labour and preterm birth;[81] (4) pregnant women with intra-amniotic infection[28–30] or intrauterine inflammation (defined as an elevation of AF concentrations of cytokines[82,83] and matrix degrading enzymes[84]) in the mid-trimester are at risk for subsequent preterm birth; (5) antibiotic treatment of ascending intrauterine infections can prevent preterm birth in experimental models of chorioamnionitis;[58,85] and (6) treatment of asymptomatic bacteriuria prevents preterm birth.[86,87]

Infection versus inflammation

Microbiological studies suggest that infection may account for 25–40% of preterm birth.[49,88] Infection is difficult to detect due to the limitations of standard microbiological techniques (cultivation of microorganisms in the laboratory) and the difficulties in obtaining an informative sample (AF requires amniocentesis). Since infection is a major cause of inflammation, we often refer to women with proven infection and those with histological evidence of acute chorioamnionitis or elevated proinflammatory cytokines in the AF as belonging to an 'inflammatory cluster'.

The frequency and clinical significance of intrauterine infection

Intrauterine infections caused by bacteria are considered to be the leading cause of infection-associated preterm birth. The amniotic cavity is considered sterile, as less than 1% of women not in labour at term will have bacteria in the AF. The isolation of bacteria in the AF is a pathological finding, which we have defined as microbial invasion of the amniotic cavity (MIAC). Most of these infections are subclinical in nature and

cannot be detected without AF analysis. The frequency of MIAC depends on the clinical presentation and gestational age. In women with preterm labour and intact membranes, the rate of positive AF cultures is 12.8%.[49] However, among those women in spontaneous preterm labour with intact membranes who deliver preterm, the frequency is 22%. Among women with PPROM, the rate of positive AF cultures at admission is 32.4%;[49] however, at the time of the onset of labour, as many as 75% of women will have MIAC,[89] suggesting that microbial invasion occurs during the latency period.

The frequency of MIAC among women presenting with the clinical picture of cervical insufficiency is up to 51%.[90,91] If the cervix is short (as determined by a sonographic cervical length of less than 25 mm), MIAC occurs in 9% of women.[92] Finally, the frequency of MIAC in twin gestations with preterm delivery is 11.9%.[93] Of interest, in twin gestations in which MIAC is detected, the presenting sac is frequently involved, while the other amniotic cavity may not have MIAC.[94]

Women with MIAC are more likely to deliver preterm, have spontaneous rupture of the membranes, develop clinical chorioamnionitis, and have adverse perinatal outcome than those with preterm labour or PPROM with sterile AF.[95] An interesting and consistent observation is that the lower the gestational age at presentation (preterm labour with intact membranes or PPROM), the higher the frequency of positive AF cultures.[96,97]

Microbiology of intrauterine infection

The most common microorganisms found in the amniotic cavity are genital *Mycoplasma* species and, in particular, *Ureaplasma urealyticum*.[48,98] Other microorganisms found in the amniotic cavity include *Streptococcus agalactiae*, *Escherichia coli*, *Fusobacterium* species, and *Gardnerella vaginalis*.[48] With the use of molecular microbiological techniques, organisms normally found in the oral cavity have been detected in the AF of women in preterm labour.[99] This observation raises questions as to the pathway used by these organisms to reach the amniotic cavity (see below).

Significance of MIAC detected only by molecular microbiology techniques

The prevalence of MIAC described in the preceeding sections is based on the results of standard microbiological methods (i.e. cultivation techniques). A positive culture can only be obtained if the culture conditions in the laboratory are able to support the growth of a particular microorganism. Since the growth requirements of all microorganisms are unknown, a negative culture cannot be taken to definitively exclude the presence of microorganisms. In other words, while a positive culture is indicative of MIAC, a negative culture indicates that the laboratory was not able to grow bacteria from the specimen, either because bacteria were absent (a true-negative result) or because the laboratory conditions did not support the growth of a specific microorganism (a false-negative result). It is noteworthy that only 1% of the whole microbial world can be detected by cultivation techniques ('the great plate count anomaly').[100–102]

Consequently, the frequency of MIAC reported previously in the literature, using cultivation techniques, represents minimum estimates. These figures are likely to change with the introduction of more sensitive methods for microbial recovery and identification. Several investigators have shown that the prevalence of MIAC is higher when molecular microbiological techniques are used to detect conserved sequences in prokaryotes (e.g. bacterial 16S ribosomal DNA with polymerase chain reaction [PCR]) or specific probes.[103–106]

The clinical significance of MIAC detected purely by molecular microbiology techniques, but not by cultivation techniques, has been recently addressed. Women with a positive PCR for *U. urealyticum*, but a negative culture, have similar adverse outcomes to women with a positive AF culture and have worse outcomes than those with sterile AF and a negative PCR.[107,108] Women with a positive PCR, but a negative culture, have the same degree of inflammation (AF interleukin [IL]-6, histological chorioamnionitis or funisitis) as those with a positive AF culture.[108] Collectively, this evidence suggests that the presence of microbial footprints detected by PCR is associated with adverse outcomes.

Intrauterine infection can also be present without a positive AF culture for microorganisms or a positive PCR. If the infection is localised to the decidua or to the space between the amnion and the chorion, microorganisms may not be detected in the amniotic cavity.[97] There is evidence that the rate of microbial colonisation in the chorioamniotic space is higher than that observed in the amniotic cavity.[97] Women with positive cultures in the membranes, but negative cultures in the AF, often have elevated AF concentrations of inflammatory markers such as IL-6.[97] Some women with intra-amniotic inflammation, but negative cultures in the AF, may have intrauterine infection in the extra-amniotic space.

Microorganisms in the chorioamniotic membranes—is it always pathological?

The amniotic cavity is normally considered sterile for bacteria, even with the use of molecular microbiological techniques. In contrast, fluorescence *in situ* hybridisation with a DNA probe specific for conserved regions of bacterial DNA (the 16S ribosomal RNA) has detected bacteria in the fetal membranes of up to 70% of women undergoing elective caesarean section at term.[109] Bacteria are often present in the membranes of women in preterm labour and intact membranes and in women with PPROM. These findings suggest that the presence of bacteria alone is not sufficient to cause preterm labour and preterm birth and that microbial

colonisation of the chorioamniotic membranes may not always elicit a fetal or maternal inflammatory response.[109]

MIAC as a chronic process

Although chorioamnionitis is traditionally considered an acute process, evidence that MIAC exists for an extended period of time is mounting. Cassell *et al.*[28] were the first to report the recovery of genital *Mycoplasma* species from 6.6% (4/61) of AF samples collected by amniocentesis between 16 and 21 weeks of gestation. Two women had positive cultures for *M. hominis* and two had positive cultures for *U. urealyticum*. Women with *M. hominis* delivered at 34 and 40 weeks without neonatal complications, while those with *U. urealyticum* had a preterm birth, neonatal sepsis and neonatal death at 24 and 29 weeks of gestation. Subsequently, Gray *et al.*[29] reported a 0.37% prevalence (9/2461) of positive cultures for *U. urealyticum* in AF samples obtained during second-trimester genetic amniocentesis. After exclusion of a therapeutic abortion case, all women (8/8) with positive AF cultures had either a fetal loss within 4 weeks of amniocentesis ($n = 6$) or preterm birth ($n = 2$). All had histological evidence of chorioamnionitis. These observations suggest that microbial invasion could be clinically silent in the mid-trimester of pregnancy and that pregnancy loss/preterm birth could take weeks to occur. A similar finding was reported by Horowitz *et al.*[30] who detected *U. urealyticum* in 2.8% (6/214) of AF samples obtained between 16 and 20 weeks of gestation. The rate of adverse pregnancy outcome (fetal loss, preterm birth and low birthweight) was significantly higher in women with a positive AF culture than in those with a negative culture (3/6 [50%] versus 15/123 [12%]; $P = 0.035$).

Intra-amniotic inflammation as a chronic process

High IL-6 concentrations in AF are considered a marker of intra-amniotic inflammation and are frequently associated with microbiological infection in the AF.[110–113] Romero *et al.* reported the results of a case–control study in which IL-6 determinations were conducted in stored fluid of women who had a pregnancy loss after a mid-trimester amniocentesis and a control group who delivered at term. Women who had a pregnancy loss had a significantly higher median AF IL-6 concentration than those with a normal outcome.[82] Similar findings were reported by Wenstrom *et al.*[114] Of note is that maternal serum concentrations of IL-6 were not associated with adverse pregnancy outcome.[114]

The same approach was subsequently used to test the association between markers of inflammation in mid-trimester AF of asymptomatic women and preterm birth. The concentrations of matrix metalloproteinase (MMP) 8,[84] IL-6,[85] tumour necrosis factor alpha (TNF-α),[115] and angiogenin[116] in AF obtained at the time of mid-trimester amniocentesis were significantly higher in women who subsequently delivered preterm than in those who delivered at term.

Collectively, the evidence cited above suggests that a chronic intra-amniotic inflammatory process is associated with both miscarriage and spontaneous preterm labour and preterm birth. Whether intra-amniotic inflammation can be detected noninvasively remains to be determined. Goldenberg *et al.*[117] showed that the maternal plasma concentration of granulocyte–colony-stimulating factor (G-CSF) at 24 and 28 weeks of gestation is associated with early preterm birth. To the extent that G-CSF may reflect an inflammatory process, this finding suggests that a chronic inflammatory process identifiable in the maternal compartment is associated with early preterm birth.

Pathways of intra-amniotic infection

Microorganisms may gain access to the amniotic cavity and fetus using any of the following pathways: (1) ascending from the vagina and the cervix; (2) haematogenous dissemination through the placenta (transplacental infection); (3) retrograde seeding from the peritoneal cavity through the fallopian tube; and (4) accidental introduction at the time of invasive procedures, such as amniocentesis, percutaneous fetal blood sampling, chorionic villus sampling, or shunting.[118] The most common pathway of intrauterine infection is the ascending route (Figure 2).

Accumulating evidence supports a relationship between periodontal disease and preterm labour and preterm birth.[75–80,99,119–123] The mechanism underlying this association has not been established definitively; however, there is experimental evidence that microorganisms found in the gingival crevice can be isolated from the AF, suggesting that maternal bacteraemia and transplacental passage could account for some of these infections. Indeed, a humoral fetal response has been demonstrated by Boggess *et al.*[124]

Microbial products in the amniotic cavity

The adverse events associated with microbial invasion can be due to the proliferation of intact microorganisms or bacterial products. The cell wall of Gram-negative bacteria contains lipopolysaccharide (LPS) or endotoxin. This potent agent is capable of inducing endotoxic shock and death.[125] Gram-positive bacteria lack LPS but contain peptidoglycans and lipoteichoic acid, components of the bacterial cell wall.[126] *Mycoplasmas* have products such as lipoglycans.[127] Many of the effects of microorganisms are mediated by these products, which can be released during bacterial death. Thus, even nonviable bacteria may exert deleterious effects. LPS, peptidoglycans, and lipoglycans are recognised by Toll-like receptors (TLRs) and other pattern recognition molecules, and can elicit an inflammatory response.

Bacterial endotoxin in AF was first identified in 1987.[128] Subsequently, it was found that the concentrations of these microbial products were significantly higher in women in preterm labour with ruptured membranes than in those

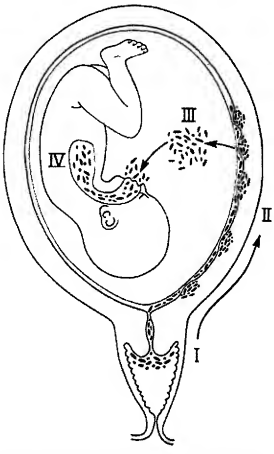

Figure 2. The most common pathway of intrauterine infection is the ascending route. (Reproduced with permission from reference 1.)

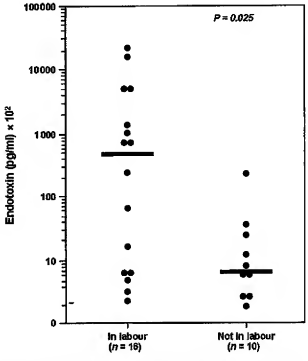

Figure 3. Concentrations of bacterial endotoxins are significantly higher in women with PPROM in labour than in those with PPROM not in labour. (Reproduced with permission from reference 129.)

with ruptured membranes but not in labour[129] (Figure 3). There is a paucity of data about the AF concentration of other microbial products. A number of experimental studies have determined that endotoxin administration into the amniotic cavity,[130] uterus,[131,132] or intraperitoneally[60,133] can result in an inflammatory response with potent biological effects in the fetal lung.[134–136] Moreover, intrauterine bacterial inoculation, in an ascending model of intra-amniotic infection, was associated with histological evidence of brain white matter damage.[137]

Inflammation as a mechanism for preterm birth

An overview of the inflammatory response

The first line of defence against infection is provided by the innate immune system. Epithelial surfaces (mucous membranes) represent the first physical barrier between the body and the microorganisms. Injuries to the epithelial surface provide a point of entry for microorganisms. These injuries can result from accidents or physiological processes (e.g. menstruation). A sexually transmitted microorganism may cause infection if it gains access to the endometrium during menstruation. Bacteria can cross intact epithelial barriers. There is experimental[138] and clinical evidence[48,128] suggesting that bacteria can cross intact fetal membranes but epithelium represents more than a physical barrier against microorganisms. Most epithelia produce natural antimicrobial peptides (e.g. alpha-defensins and beta-defensins),[139] which can kill bacteria by damaging their cell membrane.[140–143] The fetal lung produces surfactant proteins (SP-A[144,145] and SP-D[144]), which belong to the collectin family and can bind microorganisms and facilitate phagocytosis (opsonisation). Moreover, SP-A and SP-D have been shown to be involved in clearance of bacteria, fungi, and apoptotic and necrotic cells, downregulation of allergic reaction, and resolution of inflammation.[146]

Another mechanism of host defence against infection derives from the metabolic products of bacteria. Lactobacilli, which colonise the vagina shortly after birth, produce lactic acid, which lowers the pH of the vagina. This unique partnership between vaginal tissues and species-specific strains of lactobacilli has been considered responsible for enabling internal fertilisation in the evolution of mammals from amphibians.[147] In addition to the low pH, some strains of lactobacilli also produce antimicrobial products (bacteriocin-like compounds), which prevent the growth of pathogenic bacteria.[148,149]

The innate component of the immune system also provides immediate protection from microbial challenge by recognising the presence of microorganisms, preventing tissue

invasion and/or eliciting a host response to limit microbial proliferation (inflammation).[150] One of the mechanisms by which the innate immune system recognises microorganisms is by using pattern recognition receptors (PRRs), which bind to patterns of molecular structures present on the surfaces of microorganisms.[150] PRRs, which are classified according to their function and subcellular localisation, include: (1) soluble PRRs, such as 'the acute-phase proteins,' mannan-binding lectin and C-reactive protein, which act as opsonins to neutralise and clear pathogens through the complement and phagocytic systems; (2) transmembrane PRRs, which include scavenger receptors, C-type lectins, and TLRs; and (3) intracellular PRRs, including Nod1 and Nod2, retinoic-induced gene type I and melanoma differentiation associated protein 5, which mediate recognition of intracellular pathogens (e.g. viruses).[151]

Ten different TLRs have been recognised in humans.[150] TLR-4 recognises the presence of LPS (Gram-negative bacteria); TLR-2 recognises peptidoglycans, lipoproteins, and zymosan (Gram-positive bacteria, *Mycoplasmas*, and fungi); and TLR-3 recognises double-stranded RNA (viruses). The ligand for TLR-5 is flagellin.[150,152,153]

Ligation of TLRs results in activation of nuclear factor (NF)-κB, which, in turn, leads to the production of cytokines, chemokines, and antimicrobial peptides.[150] Moreover, activation of the Toll pathway also induces surface expression of costimulatory molecules required for the induction of adaptive immune responses, such as CD-80 and CD-86. In combination with antigenic microbial peptides, these molecules presented by major histocompatability complex class II proteins in dendritic cells and macrophages can activate naive CD4 T cells that initiate most adaptive immune responses.[150]

Innate immune receptors of the genital tract

TLR-1, -2, -3, -5, and -6 have been identified in epithelia from the vagina, ecto- and endocervix, endometrium, and uterine tubes.[154] Of note, TLR-4 has been shown in the endocervix, endometrium, fallopian tubes and ectocervix.[154,155] This has been interpreted as evidence that TLR-4 may participate in the modulation of the immune response in the genital tract of women and in host defence against infection.[154] Similarly, trophoblast cells can recognise and respond to pathogens through TLRs. We have shown that trophoblast cells are able to recognise pathogens through the expression of TLR-2 and TLR-4. Activation of different TLRs generates distinct trophoblast cell responses. *In vitro* studies have shown that TLR-4 ligation promotes cytokine production, while TLR-2 ligation induces apoptosis in first trimester trophoblast cells.[156] These findings suggest that a pathogen, through TLR-2, may directly promote trophoblast cell death[156] which is observed in a number of pregnancy complications including miscarriage,[157] intrauterine growth restriction,[158,159] and pre-eclampsia.[159]

The importance of TLRs in preterm parturition

Since TLRs are crucial for the recognition of microorganisms, it could be anticipated that defective signalling through this PRR will impair bacteria-induced preterm labour. A strain of mice that has a spontaneous mutation for TLR-4 is less likely to deliver preterm after intrauterine inoculation of heat-killed bacteria or LPS administration than wild-type mice.[131,160] In pregnant women, TLR-2 and TLR-4 are expressed in the amniotic epithelium.[161] Moreover, spontaneous labour at term or preterm with histological chorioamnionitis, regardless of the membrane status (intact or ruptured), is associated with an increased mRNA and protein expression of TLR-2 and TLR-4 in the chorioamniotic membranes.[161] These observations suggest that the innate immune system plays a role in parturition.

The role of proinflammatory cytokines (IL-1 and TNF-α)

Strong evidence supports a role for inflammatory mediators in the mechanisms of preterm parturition. Major attention has been focused on the role of proinflammatory cytokines such as IL-1β, TNF-α, and IL-8. Other proinflammatory and anti-inflammatory cytokines may also play a role, as can chemokines, platelet-activating factor, prostaglandins, and other inflammatory mediators. The current view is that during the course of ascending intrauterine infection, microorganisms may reach the decidua, where they can stimulate a local inflammatory reaction and the production of proinflammatory cytokines and inflammatory mediators (platelet-activating factor, prostaglandins, leucotrienes, reactive oxygen species, NO, etc.). If this inflammatory process is not sufficient to signal the onset of labour, microorganisms can cross intact membranes into the amniotic cavity, where they can also stimulate the production of inflammatory mediators by resident macrophages and other host cells. Finally, microorganisms that gain access to the fetus may elicit a systemic inflammatory response syndrome, characterised by increased concentrations of IL-6[38,39] and other cytokines,[162,163] as well as cellular evidence of neutrophil and monocyte activation.[164]

A solid body of evidence indicates that cytokines play a central role in the mechanisms of inflammation/infection-induced preterm parturition.[81,165–177] IL-1 was the first cytokine to be implicated in the onset of spontaneous preterm labour associated with infection.[165] Evidence in support of the participation of IL-1 includes: (1) IL-1 is produced by human decidua in response to bacterial products;[178] (2) IL-1 stimulated prostaglandin production by human amnion and decidua;[179] (3) IL-1 concentration and bioactivity was increased in the AF of women with preterm labour and infection;[180] (4) IL-1 could stimulate myometrial contractions[181] (C. Bulletti, pers. comm.); and (5) administration of IL-1 to pregnant animals induced preterm labour and preterm birth,[182] a phenomenon that could be blocked by the administration of its natural antagonist: the IL-1 receptor antagonist (IL-1ra).[183]

The evidence supporting the role of TNF-α in the mechanisms of preterm parturition includes: (1) TNF-α stimulates prostaglandin production by the amnion, decidua, and myometrium;[47] (2) human decidua can produce TNF-α in response to bacterial products;[184,185] (3) AF TNF-α bioactivity and immunoreactive concentrations are elevated in women in preterm labour and with intra-amniotic infection;[186] (4) in women with PPROM and intra-amniotic infection, TNF-α concentrations are higher in the presence of labour;[186] (5) TNF-α can stimulate the production of MMPs,[187,188] which may play a role in membrane rupture[189–191] and cervical ripening;[187,192,193] (6) TNF-α application on the cervix induces changes that resemble cervical ripenings;[194] and (7) TNF-α is involved in the mechanisms of bacterial-induced preterm parturition in animal models.[195,196]

Redundancy in the cytokine network

Other cytokines (IL-6,[97,110,197–200] IL-10,[181,201,202] IL-16,[203] IL-18,[204] colony-stimulating factors,[117,205,206] and macrophage migration inhibitory factor[207]) and chemokines (IL-8,[206,208–210] monocyte chemotactic protein-1,[211] epithelial-cell-derived neutrophil-activating peptide-78,[212] and regulated on activation normal T-cell expressed and secreted[213]) have also been implicated in the mechanisms of disease in preterm labour and preterm birth. The redundancy of the cytokine network implicated in parturition is such that the blockade of a single cytokine is insufficient to prevent preterm birth in the context of infection. Preterm labour can occur in knockout (KO) mice for the IL-1 type I receptor after exposure to bacteria, suggesting that IL-1 administration is sufficient, but not necessary, for the onset of birth in the context of infection.[214] However, blockade of both IL-1 and TNF-α signalling in a double KO mice model has been associated with a decreased rate of preterm birth after bacterial inoculation.[215] This is compelling evidence of the importance of IL-1 and TNF-α in the mechanisms of preterm parturition associated with infection.

Anti-inflammatory cytokines and preterm labour

IL-10 is believed to be a key cytokine for the maintenance of pregnancy. IL-10 production is significantly reduced in the placenta at term without labour compared with that in first- and second-trimester tissues, suggesting that downregulation of IL-10 is a physiological event that favors an inflammatory state around the time of the onset of labour.[201] IL-10 has also been implicated in the control of preterm parturition associated with inflammation.[202] Indeed, IL-10 expression was reduced in the placental tissues of pregnancies complicated by preterm labour and chorioamnionitis when compared with that in placental tissues from normal controls.[202] IL-10 inhibits cyclooxygenase type 2 (COX-2) mRNA expression in cultured placental explants from women following preterm labour and preterm birth but not in those from women in labour at term, indicating that the mechanisms involved in the regulation of the inflammatory response during term and preterm parturition may be different.[202] Further evidence that IL-10 plays a role in downregulation of the inflammatory response in preterm labour derives from a study in which pregnant rhesus monkeys ($n = 13$) were allocated to one of three groups: (1) intra-amniotic IL-1β infusion with maternal dexamethasone intravenously ($n = 4$); (2) intra-amniotic IL-1β + IL-10 ($n = 5$); or (3) intra-amniotic IL-1β administered alone ($n = 5$). Dexamethasone and IL-10 treatment significantly reduced IL-1β-induced uterine contractility ($P < 0.05$). The concentrations of TNF-α and leukocyte counts in AF were also attenuated by IL-10 treatment ($P < 0.05$).[181] The administration of IL-10 in animal models of infection has been associated with improved pregnancy outcome.[216,217]

Fetal involvement

The most advanced and serious stage of ascending intrauterine infection is fetal infection. The overall mortality rate of neonates with congenital neonatal sepsis ranges between 25 and 90%.[218–222] The wide range of results may reflect the effect of gestational age on the likelihood of survival. One study, which focused on infants born before 33 weeks of gestation, found that the mortality rate was 33% for those infected and 17% for noninfected fetuses.[222] Carroll *et al.*[29] have reported that fetal bacteraemia is present in 33% of fetuses with positive AF culture and 4% of those with negative AF culture, indicating that subclinical fetal infection is far more common than traditionally recognised.

Inflammation and fetal injury: the fetal inflammatory response syndrome

While the traditional definition of inflammation describes 'localised inflammation' to a particular tissue, it is now recognised that inflammation may be present in the systemic circulation. Such a state is referred to as the 'systemic inflammatory response syndrome'. This condition was originally described in adults and is often referred to by the acronym 'SIRS'. SIRS was introduced in 1992 by the American College of Chest Physicians and the Society of Critical Care Medicine to describe a complex set of findings, which often involved cardiovascular abnormalities believed to be the result of systemic activation of the innate immune system.[223] The changes, which are characterised by fever, tachycardia, hyperventilation, and an elevated white blood cell count,[223] have been attributed to the effects of cytokines and other proinflammatory mediators.[224] In 2001, the same organisation noted that the elevation of certain mediators, such as IL-6, may be associated with SIRS and this observation may bring about a new definition of the syndrome in adults, as the clinical and laboratory findings originally proposed to characterise SIRS were nonspecific.[225] We defined the fetal counterpart of SIRS, the 'fetal inflammatory response

syndrome' (FIRS), for the first time in 1997, using precisely the same parameter that was proposed in adults: an elevated IL-6 concentration (in fetal blood).[36,226]

FIRS was originally described in pregnancies complicated by preterm labour and PPROM and was operationally defined as a fetal plasma IL-6 concentration of > 11 pg/ml. Fetuses with FIRS had a higher rate of severe neonatal morbidity (e.g. respiratory distress syndrome, suspected or proved neonatal sepsis, pneumonia, bronchopulmonary dysplasia, intraventricular haemorrhage, periventricular leucomalacia, or necrotising enterocolitis)[38] and a shorter cordocentesis-to-delivery interval.[38,39] The original work describing FIRS was based on fetal blood samples obtained by cordocentesis.[38,39] Many of the findings have since been confirmed by studying umbilical cord blood at the time of birth, including the elevation of proinflammatory cytokines and the relationship between these cytokines and the likelihood of clinical and suspected sepsis.[227–229] Pathological examination of the umbilical cord is an alternative approach to determine whether fetal inflammation was present before birth. Funisitis and chorionic vasculitis are the histopathological hallmark of FIRS.[230] Funisitis is associated with endothelial activation, a key mechanism in the development of organ damage,[231] and neonates with funisitis are at increased risk for neonatal sepsis[232] and long-term handicaps, such as bronchopulmonary dysplasia[227] and cerebral palsy.[233] Another approach to detect FIRS is to measure C-reactive protein concentration in umbilical cord blood, which has been shown to be elevated in women with AF infection, funisitis, and congenital neonatal sepsis.[234] Since neutrophils in the AF are predominantly of fetal origin,[235] the AF white blood cell count can also be used as an indirect index of fetal inflammation.[235] Intra-amniotic inflammation is a risk factor for impending preterm birth and adverse perinatal outcome in women with PPROM, even in the absence of documented intra-amniotic infection.[236]

Among women with PPROM, elevated fetal plasma IL-6 level is associated with the impending onset of preterm labour, regardless of the inflammatory state of the AF (Figure 4).[39] This suggests that the human fetus plays a role in initiating the onset of labour. Maternal–fetal cooperation must occur for birth to be completed. Fetal inflammation has been linked to the onset of labour in association with ascending intrauterine infection. However, systemic fetal inflammation may occur in the absence of labour when the inflammatory process does not involve the chorioamniotic membranes and decidua. Such instances may take place in the context of hematogenous viral infections or other disease processes (e.g. rhesus alloimmunisation).

Gene–environment interaction

A gene–environment interaction is said to be present when the risk of a disease (occurrence or severity) among individuals exposed (to both genotype and an environmental factor) is greater or lower than that which is predicted from the presence of either the genotype or the environmental exposure.[237,238] The most powerful evolutionary force shaping the development of the immune system is the microbial–host interaction. Bacterial vaginosis (BV) is a risk factor for spontaneous preterm delivery.[239–241] However, meta-analysis and randomised clinical trials of antibiotic administration to prevent preterm birth have yielded contradictory results.[239–251] Macones *et al.*[252] recently reported the results of a case–control study in which participants were women who experienced spontaneous preterm labour and preterm birth and controls were women who delivered after 37 weeks. The environmental exposure was clinically diagnosed BV (symptomatic vaginal discharge, a positive whiff test, and clue cells on a wet preparation). The genotype of interest was TNF-α allele 2, given that carriage of this genotype had been shown by the authors to be associated with spontaneous preterm labour and preterm birth in previous studies.[253] The key observations were that: (1) clinically diagnosed BV was not associated with an increased risk for preterm birth (OR 1.6, 95% CI 0.8–3.5); and (2) women who carried the TNF-α allele 2 were also not at an increased risk for preterm birth (OR 1.8, 95% CI 1–3.1). In contrast, women with both BV and the TNF-α allele 2 had an odds ratio of 10 (95% CI 4.4–24) for spontaneous preterm labour and preterm birth, suggesting that a gene–environment interaction predisposes to preterm birth.[254] Similar interactions may determine the susceptibility to intrauterine infection, microbial invasion of the fetus, and the likelihood of fetal injury. Gene-to-gene interactions may also play a role in modulating the inflammatory response, and therefore, may also play a role in preterm labour and delivery.

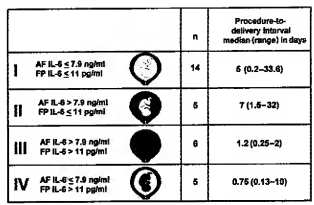

Figure 4. Classification and procedure-to-delivery interval of women according to AF and fetal plasma (FP) IL-6 concentrations. Analysis restricted to 30 women with available AF. *White* in the fetal or AF compartment represents a low FP or AF IL-6 concentration, respectively. *Black* in the fetal or AF compartment denotes elevated fetal plasma or AF IL-6 concentration, respectively. (Reproduced with permission from reference 39).

Uteroplacental ischaemia

Women in spontaneous preterm labour can be classified into two groups: those with inflammatory lesions of the placenta and membranes and those without evidence of inflammation.[255] A major challenge has been to identify the mechanisms of disease responsible for preterm parturition in the noninflammatory group.

The most common pathological features in the placenta of women who belong to the noninflammatory group are maternal and fetal vascular lesions.[255] Maternal lesions observed in the placenta of patients with a spontaneous preterm delivery include failure of physiological transformation of the myometrial segment of the spiral arteries, atherosis, thrombosis of the spiral arteries (a form of decidual vasculopathy), and a combination of these lesions. Fetal lesions may include a decrease in the number of arterioles in the villi and fetal arterial thrombosis.

Maternal vascular lesions could lead to preterm labour by causing uteroplacental ischaemia. Several lines of evidence support a role for uteroplacental ischaemia as a mechanism of disease leading to preterm labour: (1) experimental studies designed to generate a primate model for pre-eclampsia by causing uterine ischaemia showed that a proportion of animals had spontaneous preterm labour and preterm birth;[256] (2) vascular lesions in decidual vessels attached to the placenta have been reported by Arias *et al.*[257] in 34% of women in spontaneous preterm labour and intact membranes, in 35% of those with PPROM, and in only 12% of control women (term gestation without complications). Placental vascular lesions in the decidual vessels of the placenta are associated with a mean odds ratio of 3.8 and 4 for preterm labour with intact membranes and PPROM, respectively; (3) abruptio placenta, a lesion of vascular origin, is more frequent in women who deliver preterm with intact membranes[257,258] or with rupture of membranes than in those who deliver at term;[259–261] (4) women in preterm labour with intact membranes and those with PPROM who delivered preterm have a higher percentage of failure of physiological transformation in the myometrial segment of the spiral arteries than women who deliver at term;[262,263] (5) women presenting with preterm labour and intact membranes, who have an abnormal uterine artery Doppler velocimetry, are more likely to deliver preterm than those with normal Doppler velocimetry.[264,265] These results are similar to those reported by other investigators studying women before the onset of labour;[266] and (6) the frequency of small-for-gestational-age infants is increased in women delivered after preterm labour with intact membranes and preterm PROM.[32–37] Vascular lesions leading to compromise of the uterine supply line could account for both intrauterine growth restriction and preterm labour.

The precise mechanisms responsible for the onset of preterm parturition in women with uteroplacental ischaemia have not been determined. A role for the renin–angiotensin system has been postulated as the fetal membranes are endowed with a functional renin–angiotensin system,[267] and uterine ischaemia increases the production of uterine renin.[268,269] Angiotensin II can induce myometrial contractility directly[270] or through the release of prostaglandins.[271] When uteroplacental ischaemia is severe enough to lead to decidual necrosis and haemorrhage, thrombin may activate the common pathway of parturition. Evidence in support of this includes: (1) decidua is a rich source of tissue factor, the primary initiator of coagulation;[272] (2) intrauterine administration of whole blood to pregnant rats stimulates myometrial contractility,[273] while heparinised blood does not (heparin blocks the generation of thrombin);[273] (3) fresh whole blood stimulates myometrial contractility *in vitro*, and this effect is partially blunted by incubation with hirudin, a thrombin inhibitor;[273] (4) thrombin stimulates myometrial contractility in a dose-dependent manner;[273] (5) thrombin stimulates the production of MMP-1,[274] urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) by endometrial stromal cells in culture;[275] MMP-1 can digest collagen directly, while uPA and tPA catalyse the transformation of plasminogen into plasmin, which in turn can degrade type III collagen and fibronectin,[276] important components of the extracellular matrix in the chorioamniotic membranes;[277] (6) thrombin/antithrombin (TAT) complexes, markers of *in vivo* generation of thrombin, are increased in the plasma[278] and AF[279] of women in preterm labour and with PPROM; (7) an elevation of plasma TAT complex concentration in the second trimester is associated with subsequent PPROM;[280] (8) the presence of retroplacental hematoma detected by ultrasound examination in the first trimester is associated with adverse pregnancy outcomes, including preterm birth and fetal growth restriction;[281] and (9) the presence of vaginal bleeding in the first or second trimester is associated with preterm birth and other adverse perinatal outcomes.[282–284]

Fetal vascular lesions (i.e. abnormal development due to defective angiogenesis or fetal thrombosis) have not been studied as thoroughly as maternal vascular lesions, but they could lead to fetal compromise and preterm labour. One study has reported that fetuses in preterm labour with an elevated umbilical systolic/diastolic ratio are more likely to deliver preterm.[265] These results have not been confirmed.[266,285]

Although some investigators have proposed that fetal hypoxaemia may be a cause of preterm labour, studies with cordocentesis have indicated that fetal hypoxaemia and metabolic acidaemia are not more frequent in women in preterm labour and with intact membranes who deliver preterm than in those who deliver at term.[286] Similarly, Carroll *et al.*[287] have shown that fetal hypoxaemia is rare in women with PPROM. Uterine ischaemia should not be equated with fetal hypoxaemia, and no evidence currently shows that fetal hypoxaemia is a cause of preterm parturition.

Uterine overdistension

Women with mullerian duct abnormalities,[288] polyhydramnios,[289,290] and multiple pregnancy[291] are at increased risk for spontaneous preterm labour and preterm birth. Intra-amniotic pressure remains relatively constant throughout gestation despite the growth of the fetus and placenta.[292,293] This has been attributed to progressive myometrial relaxation due to the effects of progesterone[294] and endogenous myometrial relaxants such as nitric oxide.[295] Stretching can, however, induce increased myometrial contractility,[296] prostaglandin release,[297] expression of gap junction protein or connexin-43,[298] and increased oxytocin receptor in pregnant and nonpregnant myometrium.[299] The stretch-induced contraction-associated protein gene expression during pregnancy is inhibited by progesterone.[298] The effect of stretch increases in late gestation and is maximal during labour as a consequence of the relative reduction in uterine growth compared with fetal growth and of the declining circulating and/or local concentrations of progesterone.[298,300,301] The effect of mechanical forces on muscle has been studied extensively in myocardium,[302] vascular smooth muscle,[303] bladder,[304] and gastrointestinal smooth muscle[305] but not in myometrium.

Mechanical stress induces activation of integrin receptors,[306] stretch-activated calcium channels,[305,307] phosphorylation of platelet-derived growth factor receptor,[308] and activation of G proteins.[308,309] Once mechanical force is sensed, it leads to activation of protein kinase C and mitogen-activated protein kinases, increased gene expression of c-fos and c-jun, and enhanced binding activity of transcription factor activator protein-1.[310–315] Other effects of physical forces relevant to myometrium include increased expression of prostaglandin H synthase 2,[316] superoxide dismutase, and nitric oxide synthase. The nature of force/pressure-sensing mechanisms of the myometrium has yet to be determined. A role for integrins and their ligands has been proposed for other organs.[317,318] Stretch may not only induce increased myometrial contractility but may also modify the contractile response through 'mechanoelectrical feedback' similar to the one reported in the heart.[319]

The chorioamniotic membranes are distended by 40% at 25–29 weeks of gestation, 60% at 30–34 weeks of gestation, and 70% at term.[320] Stretching of the membranes *in vitro* induces histological changes characterised by elongation of the amnion cells and increased production of collagenase activity and IL-8,[321,322] while stretching of amnion cells in culture results in increased production of prostaglandin E_2.[323] Recent studies using an *in vitro* cell culture model for fetal membrane distension revealed upregulation of IL-8 and pre-B-cell colony-enhancing factor.[324] When fetal membrane explants were distended in an *in vitro* distension device to mimic the situation *in vivo*, and the gene expressions of distended explants were compared with that of nondistended explants, three genes, namely IL enhancer binding factor 2, huntingtin-interacting protein 2, and interferon-stimulated gene encoding a 54 kDa protein, were found to be upregulated.[325] Collectively, these observations suggest that mechanical forces associated with uterine overdistention may result in activation of mechanisms leading to membrane rupture. Premature cervical ripening is also a feature of women with multiple gestations and those with certain mullerian duct anomalies (e.g. incompetent cervix in diethylstilbestrol [DES]-exposed daughters). IL-8,[194,326–328] MMP-1,[329] prostaglandins,[330–332] and nitric oxide[333] have been implicated in the control of cervical ripening. Inasmuch as these mediators are produced in response to membrane stretch, they may exert part of their biological effects in parturition by stimulating extracellular matrix degradation of the cervix.

Several lines of evidence indicate that women with twins and higher order multiple pregnancies also represent a heterogeneous group. Some women suffer preterm labour associated with MIAC.[93,94,334] Others may have abnormalities of trophoblast invasion leading to vascular pathology with and without fetal growth disorders. These separate mechanisms of disease may operate in conjunction with uterine overdistension to activate the components of the common terminal pathway.

Abnormal allograft reaction

The fetoplacental unit has been considered nature's most successful 'graft'. Reproductive immunologists have suggested that abnormalities in the recognition and adaptation to a set of foreign antigens (fetal) may be a mechanism of disease responsible for recurrent pregnancy loss, intrauterine growth restriction, and pre-eclampsia.[335–338] Chronic villitis of unknown aetiology has been proposed to be a lesion akin to 'placental rejection'. The presence of these lesions in a subset of women who deliver after spontaneous preterm labour provides indirect support for the concept that immune abnormalities may be responsible for preterm labour. We have observed that some women in preterm labour, in the absence of demonstrable infection, have elevated concentrations of the IL-2-soluble receptor.[255] Elevated plasma concentrations of the IL-2 receptor are considered an early sign of rejection in women with renal transplants.[339] Further studies are required to define the frequency and clinical significance of this pathological process in preterm labour. It is noteworthy that the traditional view of the fetus as an allograft has recently been challenged.[340] The normal relationship between the mother and the conceptus has been likened to that of invertebrate allorecognition, in which cytotoxicity and rejection reactions are not inevitable consequences of exposure to foreign antigens. Pathological processes resulting from activation of the effector limb of the immune response (i.e. natural killer cells,

macrophages, etc.) could still play an important role in the pathophysiology of preterm labour.

Over 10 years ago, Holmes proposed that a complement-mediated mechanism was required for fetal survival in humans.[341–343] The complement system is a group of proteins that are activated during an inflammatory response triggered by foreign invaders. Recent studies in mice support the hypothesis that some components of the innate limb of the immune response may be suppressed during normal pregnancy.[344,345] In mice, a cell surface protein called Crry suppresses the complement system, and its expression is essential for the survival of the embryo during pregnancy.[345] When the Crry gene was inactivated (KO experiments), none of the Crry-deficient embryos lived to term (they died *in utero*). The dead embryos had a massive invasion of inflammatory cells and showed evidence of activated complement system in trophoblasts. Pregnant mice that were a complement-deficient strain gave birth to normal pups. These experiments suggest that the lack of Crry gene expression in trophoblasts led to activation of the complement system and inflammatory cells, which in turn destroyed the trophoblasts and the embryo. Although there is no gene structurally homologous to the Crry gene, humans have two other complement regulators, namely decay-accelerating factor and membrane cofactor protein, which are likely to serve the same functions as Crry in mice.[341–350] A role for complement C5a receptors and neutrophils in fetal injury has been recently proposed in the antiphospholipid antibody syndrome.[351] Whether or not this mechanism is operative in some women with preterm labour remains to be determined.

Allergic phenomena

Another potential mechanism for preterm labour and preterm birth is an immunologically mediated phenomenon induced by an allergic mechanism. We have previously proposed that an allergic-like immune response (type I hypersensitivity) may be associated with preterm labour.[352] The term 'allergy' refers to disorders caused by the response of the immune system to an otherwise innocuous antigen.[353] The antigen (allergen) cross-links immunoglobulin E (IgE) bound to high-affinity receptors on mast cells, causing degranulation of these cells and the initiation of inflammation.[354] The required components of an allergic reaction are: (1) allergen; (2) production of IgE by B cells (Th2); and (3) the effector system composed of mast cells and the target organ mediators released by these cells, such as bronchial smooth muscle for asthma or smooth muscle in the gastrointestinal tract for food allergies.[352]

The evidence suggesting that an allergic-like phenomenon may operate in preterm labour is the following: (1) the human fetus is exposed to common allergens (i.e. house dust mite) as this compound has been detected in both AF in the mid-trimester of pregnancy and fetal blood. Moreover, the concentrations of the allergen are higher in fetal blood than in maternal blood;[355] (2) allergen-specific reactivity has been shown in umbilical cord blood at birth and as early as 23 weeks of gestation;[356] (3) pregnancy is considered a state in which there is preponderance of a Th2 cytokine response that favors the differentiation of naive CD4$^+$ T cells to the Th2 phenotype with increased capacity for cytokine secretion in the IL-4 gene cluster and this predisposes to a switch to IgE production by B cells; (4) the uterus is a rich source of mast cells—the effector cells of allergic-like immunological reactions;[357] (5) several products of mast cell degranulation can induce myometrial contractility (i.e. histamine and prostaglandins);[358,359] (6) pharmacological degranulation of mast cells with a compound called '48/80' induces myometrial and cervical contractility;[360,361] (7) incubation of myometrial strips from sensitised and nonsensitised animals with an anti-IgE antibody increases myometrial contractility;[360] (8) human myometrial strips obtained from women known to be allergic to ragweed show increased myometrial contractility when challenged *in vitro* by the allergen (RE Garfield, pers. comm.). Moreover, sensitivity of the myometrial strips of nonallergic women can be transferred passively by preincubation of the strips with human serum (RE Garfield, pers. comm.); (9) nonpregnant guinea pigs sensitised with ovoalbumin and then challenged with this antigen show increased uterine tone;[360] (10) traditional descriptions of animals dying of anaphylactic shock show enhanced uterine contractility when an autopsy was performed immediately after death; (11) severe latex allergy in a pregnant woman after vaginal examination with a latex glove was followed by regular uterine contractions;[362] (12) human decidua contains immune cells capable of identifying local foreign antigens, including macrophages, B cells, T cells,[363,364] and dendritic cells;[365] and (13) we have identified a subgroup of women in preterm labour who have eosinophils in the AF as the predominant white blood cell.[352] Under normal circumstances, white blood cells are not present in AF. The presence of eosinophils, therefore, suggests an abnormal immune response and perhaps they are the markers of an allergic-like response in preterm labour. The antigen eliciting an abnormal immunological response remains to be identified. Recent evidence suggests that administration of ovoalbumin to sensitised pregnant guinea pigs can induce preterm labour and preterm birth and that this phenomenon can be prevented with treatment with antihistaminics.[366]

Cervical disorders

Although cervical insufficiency is traditionally considered a cause of mid-trimester abortion, accumulating evidence suggests that a wide spectrum of disease exists.[367] This spectrum includes the well-recognised recurrent pregnancy loss in the mid-trimester, some forms of preterm labour (presenting

with bulging membranes in the absence of significant uterine contractility or rupture of membranes), and probably precipitous labour at term. Cervical disease may be the result of a congenital disorder (i.e. hypoplastic cervix or DES exposure *in utero*), surgical trauma (i.e. conisation resulting in substantial loss of connective tissue), or traumatic damage to the structural integrity of the cervix (i.e. repeated cervical dilatation associated with termination of pregnancy).[368] Cervical insufficiency is a syndrome in which the predominant feature is cervical ripening. Some cases of cervical insufficiency in the mid-trimester may be caused not by a primary cervical disease leading to premature ripening but by another pathological process, such as infection. Intrauterine infection has been shown in nearly 50% of women with a clinical presentation consistent with acute cervical insufficiency.[90] The reader is referred to a detailed review of cervical insufficiency and the role of cervical cerclage in the prevention of preterm birth recently published by the authors.[369]

Hormonal disorders

Progesterone is central to pregnancy maintenance.[370] This biological effect is carried out in all the components of the common pathway of parturition. Specifically, progesterone promotes myometrial quiescence, down-regulates gap junction formation, inhibits cervical ripening, and decreases the production of chemokines (i.e. IL-8) by the chorioamniotic membranes, which is thought to be key to decidual/membrane activation.[371–374] Progesterone is considered important for pregnancy maintenance in humans because inhibition of progesterone action could result in parturition. Administration of progesterone receptor antagonists [i.e. RU486 (Mifepristone) or ZK 98299 (onapristone)] to pregnant women,[375] non-human primates,[376] and guinea pigs[372] can induce labour.[370] Thus, a suspension of progesterone action is believed to be important for the onset of labour in humans. In contrast to the effects of progesterone, estrogens increase myometrial contractility and have been implicated in the induction of cervical ripening.[371–373].

In many species, a fall in maternal serum progesterone concentration (progesterone withdrawal) occurs prior to spontaneous parturition[377] (see Table 1). However, in humans, non-human primates and guinea pigs a progesterone withdrawal is not apparent. The reader is referred to an excellent review by Young[378] for a comparative physiology of parturition in mammals.

The mechanism by which progesterone action is suspended has eluded definition. Five potential mechanisms have been invoked to explain this paradox: 1) reduced bioavailability of progesterone by binding to a high affinity protein;[379–380] 2) increased cortisol concentration in late pregnancy that may compete with progesterone for binding to the glucocorticoid receptor;[381] 3) conversion of progesterone to an inactive form within the target cell before interacting with its receptor;[382–383] 4) quantitative and qualitative changes in progesterone receptor isoforms (PR-A, PR-B, PR-C);[384–387] 5) changes in progesterone receptor co-regulators;[388] and 6) a functional progesterone withdrawal through NF-κB.[389–391] The interested reader is referred to articles on this complex subject for details.[375–399]

Progesterone actions are mediated by multiprotein complexes including progesterone receptors, modifying components (co-regulators and adaptors) and effector proteins (RNA-polymerase, chromatin-remodelling and RNA-processing factors).[388] In addition, non-genomic mechanisms have recently been proposed.[388] The specific role of progesterone in the mechanism responsible for preterm labour remains to be elucidated.

The spectrum of reproductive abnormalities associated with progesterone deficiency is broad. Luteal phase deficiency (LPD) is widely believed to be a cause of infertility[400,401] and is

Table 1. Source of steroids and mechanism for progesterone withdrawal before parturition in several species. (Reproduced with permission from reference 377.) Modified from Liggins, GC. Endocrinology of parturition. In: Novy, MJ & Resko, JA, editors. Fetal Endocrinology. New York: Academic Press; 1981. p. 211–37.

Species	Sources of steroids in late pregnancy	Fall in maternal progesterone concentration	Mechanisms
Mouse	Corpus luteum	Yes	Luteolysis
Rat	Corpus luteum	Yes	Luteolysis
Rabbit	Corpus luteum	Yes	Luteolysis
Goat	Corpus luteum	Yes	P450 c17/ Luteolysis
Cow	Placenta	Yes	P450 c 17
Sheep	Placenta	Yes	P450 c 17
Guinea-pig	Placenta	No	?
Primates	Feto-placental unit	No	?

P450 C17 enzymes (17alpha-hydroxylase and C17–20 lyases activities) are induced in the placenta by an increase in fetal cortisol concentration. Luteolysis is provoked by prostaglandin F2α acting on fetal plasma receptor.

commonly implicated in the aetiology of recurrent miscarriage.[402,403] This disorder has been defined as either a defect of progesterone secretion by the corpus luteum or a defect in endometrial response to progesterone.[404,405] In LPD, the ovary could function well enough to ovulate, but the function of the corpus luteum and/or endometrium would be below the physiological threshold sufficient for conception or pregnancy maintenance. Since there is no general agreement on the criteria for the diagnosis of LPD,[400,401,406,407] the clinical significance and contribution of LPD to adverse pregnancy outcome remain uncertain. One retrospective study including 540 infertile women with a diagnosis of LPD (endometrial biopsy was out of phase by 2 days in two consecutive cycles) showed a high rate of preterm birth (31.2%) in women who had not received progesterone treatment.[408] In contrast, women who were treated with progesterone (vaginal suppositories for at least 12 weeks) showed a lower rate of preterm delivery at less than 32 weeks and less than 37 weeks of gestation (for 32 weeks, no progesterone: 12.5% [4/32] versus progesterone: 1.3% [6/469]; $P < 0.01$, and for 37 weeks, no progesterone: 31.2% [10/32] versus progesterone: 13.7% [64/469]; $P < 0.01$).[408]

Other than primary progesterone functional deficiency (e.g. LPD), reduction in progesterone function could result from other pathological mechanisms such as intrauterine infection. In animal models of ascending intrauterine infection, bacteria-induced and LPS-induced preterm birth are preceded by a significant fall in serum progesterone concentration,[409] which was attributed to: (1) LPS- and proinflammatory cytokines-induced prostaglandin synthesis and subsequent leuteolysis; (2) direct antigonadotropic effect of proinflammatory cytokines and hence suppression of progesterone production; and (3) upregulation of inducible form of nitric oxide synthase by proinflammatory cytokines and, thus, inhibition of steroidogenesis including progesterone production. Hirsch and Muhle[410] have argued that the fall in serum progesterone concentration is unlikely to be a primary mechanism by which intrauterine inoculation with bacteria causes preterm parturition in mice since the mean interval to delivery is shorter in animals with intrauterine infection than in ovariectomised animals, despite a higher serum progesterone concentration was observed in the former.[410]

Intrauterine infection is associated with an increase in proinflammatory cytokines[169,411] (i.e. IL-1, TNF-α, etc.) in AF,[180,186,412,413] fetal membranes,[414,415] decidua,[165,184,416] and myometrium.[417–420] In the context of infection-related preterm birth, the increased concentration of IL-1β in gestational tissue could stimulate NF-κB.[389,421,422] The activation of NF-κB can increase COX-2 (mRNA and protein expression) and prostaglandin production,[389] and also could repress progesterone activity, as proposed by Allport *et al.*[391] (see above), resulting in a functional progesterone withdrawal and, thus, preterm parturition. Randomised clinical trials indicate that progesterone administration to women with a history of a previous preterm birth reduces the rate of spontaneous preterm birth.[423,424] This data is discussed in detail in another article in this supplement. The mechanisms by which progesterone administration prevent preterm birth are unknown at this time.

Pregnancy and stress

Maternal stress, of exogenous or endogenous origin, is a risk factor for preterm delivery.[425–429] The nature and timing of the stressful stimuli can range from a heavy workload to anxiety.[430,431] The stressful insult could occur during pregnancy or in the pre-conceptual period.[431–433] For example, starvation before pregnancy can lead to a spontaneous preterm delivery in sheep.[434] Though the precise mechanism whereby stress induces preterm parturition is not known, a role for CRF has been proposed.[10] Indeed, the trajectory in CRF serum concentrates identifies women at risk for preterm, term, and post-term delivery.[12] Since this hormone is produced not only by the hypothalamus, but also by the placenta, the mechanisms regulating its production have been attributed to a 'placental clock.'[12] Maternal plasma concentrations of CRF are elevated in both term and preterm parturition. Moreover, patients with increased plasma concentrations of CRF in the midtrimester are at an increased risk for preterm delivery.[12,435] The precise mechanisms by which CRF induces parturition have been subject to intensive investigation and involve the production of cortisol and prostaglandins.[435,436]

Summary

The emerging picture is that preterm labour, PPROM, and cervical insufficiency are syndromes. Multiple pathological processes may lead to myometrial contractions, membrane/decidual activation and cervical ripening. The clinical presentation (i.e. preterm labour, preterm cervical ripening without significant contractility, or PPROM) will depend on the nature and timing of the insults on the various components of the common terminal pathway. This view of preterm parturition has considerable implications for the understanding of the cellular and biochemical mechanisms responsible for the initiation of parturition, as well as the diagnosis, treatment, and prevention of preterm birth. Since preterm labour is a heterogeneous condition, it is unlikely that one treatment will prevent all cases of preterm birth in patients at risk. We consider interventions such as tocolysis, cerclage, and bedrest to represent attempted treatments for only one of the manifestations of the communal pathway of parturition (i.e. uterine contractility, membrane/decidual activation, and cervical disease) but not necessarily for the underlying pathological process responsible for this activation. For example, tocolysis can prolong pregnancy for up to 7 days,[437] which may allow for the administration of steroids, accomplish maternal transfer

to a tertiary care center, and institute other measures that may help improve pregnancy outcome (i.e. antibiotic administration to women with asymptomatic bacteriuria or other infection-related conditions). It is possible that tocolysis may reduce perinatal morbidity in a particular group of women, and research to identify such a group is urgently needed.

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